

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: ANDERSON, Norman Leigh	)	Confirmation No: 6420
	)	
Application Serial No.: 10/676, 005	)	Group Art Unit: 1655
	)	
Filed: October 02, 2003	)	Examiner: Jana A. Hines
	)	
	)	Attorney Docket: 15503-001US

For: *HIGH SENSITIVITY QUANTITATION OF PEPTIDES BY MASS SPECTROMETRY*

### Declaration under 37 C.F.R. § 1.132

I, Dr. Terry W. Pearson, declare and say:

1. I am Professor of Biochemistry and Microbiology at the University of Victoria, Victoria, B.C., Canada.

2. A copy of my *Curriculum vitae* is appended below as Appendix A. I am a site Principal Investigator on a \$15M 5 year grant from the National Cancer Institute aimed at implementing SISCAPA assays of candidate cancer biomarkers. I am also a participant in discussions regarding commercialization of the SISCAPA technology.

3. I have been making and using antibodies since 1974 when I was a post doctoral scientist at the Medical Research Council Laboratory of Molecular Biology (MRC-LMB) in Cambridge, England. I was involved in the early stages of monoclonal antibody technology with Dr. Cesar Milstein and Dr. Georges Kohler and published several research papers with them. Kohler and Milstein published their seminal paper on monoclonal antibodies in 1975 and in 1984 were both awarded the Nobel Prize in Medicine for the discovery of the principle of production of monoclonal antibodies.

4. The first monoclonal antibody ever sold commercially was one that I produced in Cambridge in 1976, long before monoclonal antibody technology had spread across the world.

5. I am in charge of antibody derivation, selection and implementation for a variety of research projects, involving immunodiagnosis of diseases such as cancer and African sleeping

sickness. I am also involved in designing and making antibody reagents for basic biomedical research. Most of my work focuses on making anti-peptide and anti-protein antibodies for a variety of uses, including immunodiagnostic assay development and measurement of host-defense peptides in human plasma.

6. Over the past 34 years much of my research has focused on identification, purification and immunochemical analysis of antigens using both polyclonal and monoclonal antibodies. This work has involved epitope mapping, development of new methods for making, screening and analyzing polyclonal and monoclonal antibodies and using antibodies for development of new immunodiagnostic tests.

7. It became clear in the 1980's that antigen identification was a limiting step in development of new diagnostic tests, followed by the requirement for accurate measurement and validation of potential biomarkers. It was already broadly known that making antibodies suitable for sensitive antigen capture ELISA diagnostic assays was fraught with difficulties, including the requirement for making antibody pairs specific to two distinct epitopes on the biomarker in question. In addition, off-target binding had to be avoided by careful selection of antibodies. The stringency required for obtaining antibody pairs suitable for clinical assays was formidable and limited the number of successful assays due to technical difficulties, coupled with the expense involved.

8. In 2002-2004, I began using mass spectrometric peptide analysis in methods for identification and quantification of proteins in complex mixtures such as human plasma. It soon became clear that enrichment of peptides would be required for detection of most peptides that are present in low amounts. Some researchers fractionated samples using a variety of ion-exchange and size exclusion chromatography and global glycopeptide enrichment methods, however these were labour-intensive and expensive techniques that resulted in only modest enrichment.

9. In 2002, there was some published work showing that a few select antibodies could be used to enrich peptides from simple protein digests followed by mass spectrometric analysis in a process called epitope mapping. This method was useful since no peptide

quantification was required and since the amino acid sequence of the parental molecule and its possible peptide fragments were known. Thus the antibody-enriched peptides could be identified using their masses, even using MALDI-TOF mass spectrometry that does not give peptide sequence information (e.g., Zhao et al, Proc. Nat. Acad. Sci. USA (1996) 93:4020-4024).

10. At the time that the captioned application was filed, there was no expectation that antibodies could be used to selectively bind and enrich typical tryptic peptides from a complex mixture such as digested human plasma. This was particularly true for peptides selected for their detection and identification characteristics in a triple quadrupole mass spectrometer, since such peptides were not considered as normal B-cell epitopes (i.e., antigens to which antibodies could be made) but rather were selected as proteotypic sequences for intact proteins. Moreover, it was widely believed that the sequences of short peptides would be repeated in a number of different proteins, and this would make them unsuitable as specific surrogates for a given protein.

11. It was both surprising and exciting to me and my colleagues involved in immuno-mass spectrometry when it was demonstrated that the combination of methods described in the captioned application (some aspects of which are set forth in the claims appearing in Appendix B) appeared to solve the problems encountered with previous methods, thus allowing a general method for protein quantitation by measurement of peptide surrogates. First, it was demonstrated that antibodies of high affinity could be made to relatively short tryptic peptides (that are not considered standard B cell epitopes). Second, by appropriate selection of antibodies, it was shown that peptides could be enriched from extremely complex mixtures and that such enriched peptides could be unequivocally identified by mass spectrometry. Third, quantification of the enriched peptides could be performed using stable isotope internal standards. The method as described and claimed is broadly and universally applicable to measurement of proteins in extremely complex mixtures.

12. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any document or any registration resulting therefrom.

Date: June 7, 2009

A handwritten signature in black ink, appearing to read "Terry W. Pearson", written over a horizontal line.

Dr. Terry W. Pearson

## Appendix A

## CURRICULUM VITAE

### TERRY WILLIAM PEARSON

Professor of Biochemistry and Immunochemistry

**Address:** Department of Biochemistry and Microbiology  
University of Victoria  
P.O. Box 3055, STN CSC  
Petch Building, office Room 250; Lab room 252  
Victoria, British Columbia, V8W 3P6  
Canada

phone:

fax:

e-mail:

**Date of Birth:** February 2, 1946

**Place of Birth:** Vernon, British Columbia

**Citizenship:** Canadian

**Marital Status:** Married with two daughters

#### Education

1964-67 B.Sc. (Hons.) First Class, Microbiology  
University of British Columbia, Vancouver  
Supervisor: Dr. Julia Levy. Thesis: Physiology of *Botulinus* toxin  
production and purification and characterization of the active  
molecule.

1968-73 Ph.D. Immunology  
University of British Columbia, Vancouver  
Supervisors: Dr. Doug Kilburn and Dr. Julia Levy. Thesis: Studies  
of the molecular requirements for lymphocyte stimulation in cellular  
immune responses.

#### Experience and Positions Held

1965-68 Research Assistant and Laboratory Instructor,  
Department of Microbiology, UBC, Vancouver, Canada

1968-73 Doctoral Research and Teaching Assistant,

Department of Microbiology, University of British Columbia,  
Vancouver, Canada

- 1974-76      Postdoctoral Fellow  
Medical Research Council, Laboratory of Molecular Biology,  
Hills Road, Cambridge, U.K.  
(Dr. Sydney Brenner, Dr. Edwin S. Lennox and Dr. Cesar Milstein)
- 1976-77      Member of the Scientific Staff  
Cell Biology Division, Medical Research Council, Laboratory of  
Molecular Biology, Hills Road, Cambridge, U.K.
- 1977-78      Member of the Scientific Staff  
International Laboratory for Research on Animal Diseases  
(ILRAD), Nairobi, Kenya
- 1978-79      Senior Scientist, Immunology  
International Laboratory for Research on Animal Diseases  
(ILRAD), Nairobi, Kenya
- 1979-83      Assistant Professor  
Department of Biochemistry and Microbiology  
University of Victoria  
Victoria, British Columbia, Canada
- 1983-88      Associate Professor  
Department of Biochemistry and Microbiology  
University of Victoria  
Victoria, British Columbia, Canada
- 1985-86      Visiting Scientist  
International Laboratory for Research on Animal Diseases (ILRAD)  
Nairobi, Kenya
- 1987          Acting Chair  
Department of Biochemistry and Microbiology  
University of Victoria  
Victoria, British Columbia, Canada
- 1988-present      Professor  
Department of Biochemistry and Microbiology  
University of Victoria  
Victoria, British Columbia, Canada
- 1990-1991      Visiting Scientist

International Laboratory for Research on Animal Diseases (ILRAD)  
Nairobi, Kenya

1996-97                      Visiting Scientist  
                                 Central Veterinary Laboratory, Windhoek, Namibia and  
                                 International Livestock Research Institute (ILRI)  
                                 Nairobi, Kenya

#### **Awards**

1964-67                      Government of British Columbia Scholarships

1965                          Knights of Pythias Scholarship

1966                          IBM Scholarship

1967                          Canadian Medical Research Council Scholarship

1970-73                      Canadian Medical Research Council Trainee

1995                          Inaugural Award for Excellence in Science Teaching  
                                 University of Victoria, Victoria BC Canada

2004                          Wardle National Award for contributions to Parasitology (Can Soc  
                                 for Zoology)

#### **Membership in Learned Societies**

1. Member of the British Society for Immunology
2. Member of the Canadian Society for Immunology
3. Member of the American Association for Advancement of Science.

#### **Research Projects (Postdoctoral, Cambridge, England)**

1. Definition of unique and common tumor-specific antigens on methylcholanthrene-induced tumors from B10 congenic mice. With: Dr. Karol Sikora, Dr. Edwin S. Lennox, and Dr. Sydney Brenner.
2. Linkage relationships between tumor-specific antigens and H-2 antigens on methylcholanthrene-induced tumors. With: Dr. Edwin S. Lennox, Dr. Karol Sikora and Dr. Sydney Brenner.
3. Regulation of alloantigen expression in hybrid cell lines: control of Thy-1 and H-2 expression in T cell-B cell hybrids. With: Dr. Georges Köhler and Dr. Cesar Milstein.



4. Cell-mediated killing of influenza virus infected tumor cells. With: Dr. Brigitte Askonas and Dr. Hans Zweerink, NIMR, Mill Hill, London and Dr. Edwin S. Lennox, MRC, Cambridge, England.
5. Production of specific monoclonal antibodies in culture. With: Dr. Giovanni Galfre, Dr. Andreas Ziegler, Dr. Georges Köhler and Dr. Cesar Milstein.

#### **Research Projects (Nairobi, Kenya, 1977-1979)**

1. Studies of immune responses to African trypanosomes. With: Dr. G.E. Roelants.
2. Studies on antigenic variation of African trypanosomes. With: Dr. T. McGuire, Dr. S. Kar and Dr. G. Roelants.
3. Analysis of bovine lymphocyte subpopulations using monoclonal antibodies. With: Dr. G. Roelants and Dr. M. Pinder.
4. High resolution analysis of complex protein mixtures using multiple two-dimensional gel electrophoresis and monoclonal antibodies. With: Dr. N. L. Anderson (Argonne, Illinois).

#### **Research Projects 1979-present (Victoria, BC)**

1. Studies on the antigenic polymorphisms of the variant surface glycoproteins of African trypanosomes.
2. Immunoregulation in trypanosomiasis.
3. Identification and structure of antigens of various life cycle stages of African trypanosomes.
4. Immunodiagnosis of African trypanosomiasis.
5. Diagnosis of gonococcal infections using monoclonal antibodies.
5. Biochemistry and immunochemistry of *Leishmania* parasites.
6. Vaccine development for *Theileria parva*
7. Development of diagnostic tests for *Kudoa thyrsites* infections of salmon.
8. Plasma proteome- diagnostic methods
9. Tsetse -trypanosome molecular interactions

10. Immuno-mass spectrometric methods for diagnosis of microbial infections

### **Research Projects: study leave and other collaborative visits to Africa**

1. Study Leave: Visiting Scientist, ILRAD, Nairobi, Kenya (July 1985 - July 1986)
  - purification of variant surface glycoprotein (VSG) from *Trypanosoma vivax*;
  - analysis of protein changes in *Theileria parva*-infected lymphoblasts.
2. Visiting Scientist, ILRAD, Nairobi, Kenya (July 1990 – October 1991)
  - cloning of growth factors in trypanosomes.
3. Study Leave: Visiting Scientist, Central Vet Lab, Windhoek, Namibia (September 1996 - January 1997)
  - antigens of mycoplasma-induced bovine lung disease.
4. Visiting Scientist, Chobe National Park, Kasane, Botswana (December 1996 - January 1997)
  - establishment of an ELISA system for diagnosis of disease in African Cape buffalo.
5. Visiting Scientist, Molecular Biology Laboratory, ILRI, Nairobi, Kenya (January 1997 – August 1997)
  - molecular mechanisms involved in lectin-induced apoptosis in African trypanosomes.

### **Current Research at UVic**

1. Molecular interactions between African trypanosomes and their tsetse fly vectors.
2. Methods for diagnosis of disease using the human plasma proteome, antibody capture affinity chromatography, peptides and mass spectrometry.

### **Membership and service on international, national and provincial professional bodies and societies**

#### **Member, Scientific Advisory Boards etc.**

Pacific Isotopes and Pharmaceuticals Ltd. (a company formed by BCDC, in conjunction with TRIUMF, UBC, Vancouver, B.C.)	1984-88
TAGO Immunochemicals, San Francisco, California (SAB member)	1982-84
Director, Science Council of B.C.	1982-86
Director, Terry Fox Medical Research Foundation	1982-87
Wardle Award Committee, Canadian Society of Zoology	2004-2006
GeneMax, Vancouver, BC. Member, Board of Directors	2005-2006

Plasma Proteome Institute, Washington DC, Senior Scientific Advisor	2006-
TapImmune Inc., Vancouver, B.C. Scientific Advisory Board	2006-
Epitomics, Inc. Burlingame, CA. Scientific Advisory Board	2007-
biOasisTechnologies, Inc. Vancouver, B.C. Member, Board of Directors	2007-
Advisor: Bioventures for Global Health, Washington DC.	2009-

### **Conference organizational committees**

Sessional Chairman, World Health Organization Workshop. *In vitro* Cultivation of Major Tropical Disease Pathogens, held at ILRAD, Nairobi, Feb. 1979.  
 Workshop Chair- Canadian Society for Immunology, Lake Louise, March, 1999  
 Workshop Chair - UNESCO Molecular Biology Training Course, ICRO, Lagos, Nigeria, May 2002

### **Grant committees**

NSERC Strategic Grants Panel Member, Ottawa, Ontario	1982-84
B.C. Health Care Research Foundation	1988-90
NSERC Animal Biology Grants Subcommittee	1990-94
B.C. Science Council, Chairman of Ad Hoc Biotechnology Committee	1984-86

### **Grant proposals reviewed**

Each year more than 10 grants reviewed (NSERC, CIHR, NIH, Wellcome Foundation)

### **Visiting scientists hosted**

More than 15 over the past 5 years (1-7 days per visit)

### **Editorships (editorial boards)**

Journal of Immunological Methods	1980-84
American Journal of Tropical Medicine and Hygiene	1984-88
Editor, Hybridoma Cell Bank for Monoclonal Antibodies	
1982-84	
Associate Editor, PLoS Neglected Tropical Diseases	2008-

### **Reviews for journals, book reviews, published commentaries**

Approximately 50 over the past 5 years (Mol. Bioc. Parasitology, Parasitology, Trends in Parasitology, Vaccine, Experimental Parasitology, PLoS Neglected Tropical Diseases)

### **Other professional activities**

**Site Reviewer**

Medical Research Council of Canada, Quebec City and Montreal	1996
International Centre of Insect Physiology and Ecology, Nairobi, Kenya	1997
International Livestock Research Institute, Nairobi, Kenya	1997
Canadian Council on Animal Care-National Level 4 lab, Winnipeg	2005
Canadian Council on Animal Care-Inflazyme, Richmond, BC	2005
Canadian Council on Animal Care-Stressgen Biotechnologies, Victoria	2006

**Consultant**

Molecular Anatomy Program, Argonne National Laboratory, US Department of Energy, Argonne, Illinois	1981-84
Monoclonal antibodies, MDS Health Group Ltd., Toronto, Ontario	1981-84
Caltag Immunochemicals, South San Francisco, California, and CBR International, Sidney, B.C.	1994-96
StemCell Technologies, Inc., Vancouver, B.C.	1996-
Canadian Council On Animal Care "Immunological Procedures"	1999
Monoclonal Antibodies, EY Laboratories, San Mateo CA	1999-
Protein detection - Large Scale Biology Corporation, Rockville MD	1999-2003

**Collaborations**

University of Bern, Switzerland	Dr. I. Roditi, Dr. P. Butikofer (trypanosome surface molecules)
ILRI, Nairobi, Kenya	Dr. P. Toye (Theileria vaccine)
Howard Hughes Laboratory, Kampala, Uganda	Dr. T. Egwang (malaria)
Yale University, New Haven, CT	Dr. S. Aksoy (tsetse-tryps)
Univ. of Liverpool Tropical Medicine	Dr. M. Lehane (tsetse tryps)
University of Alberta, Edmonton	Dr. M. Belosevic (tryps)
University of BC	Dr. R. Hancock (antimicrobial peptides-tryps)
TIGR, Bethesda, MD	Dr. V. Nene (Theileria proteomics)
Plasma Proteome Institute, Washington, DC	Dr. L. Anderson (plasma proteomics)
Stemcell Technologies, Vancouver, BC	Dr. Terry Thomas (stem cell proteomics)
Hokkaido University, Sapporo, Japan	Dr. C. Sugimoto (trypanosome diagnostics)
Obihiro University, Obihiro, Japan	Dr. N. Inoue (trypanosome proteomics)

**Other**

Faculty member, Division of Atomic Energy in Food and Agricultural Research.  
 Training course in Applications of Nuclear Techniques to Studies on pathogenesis  
 and Immunology of Parasitic Diseases in Domestic Livestock, ILRAD, Nairobi,  
 Kenya, Feb.-Mar. 1979.

Adviser, Scientific Working Group on the Immunology of Malaria, WHO-UNDP, Panama, Jun. 1979.

Faculty member, invited by World Health Organization to act as part of the faculty for a course: Monoclonal Antibodies and Their Application to Parasitic Diseases. Lausanne, Switzerland, Oct. 1979.

Chairman and Rapporteur, 2nd Annual Meeting of the Scientific Working Group on African Trypanosomes (WHO), Nairobi, Kenya, Feb. 1980.

President and Director of Research, Vancouver Island Antibodies Ltd., Victoria, B.C., 1981-present Company formed to produce antisera and monoclonal antibodies for use in biomedical research and in immunodiagnostic assays.

Adjunct Associate Professor, Dept. Lab Practice/Parasitology, School of Public Health, Univ. North Carolina, Chapel Hill, N.C., U.S.A. (continuing)

Trustee, Director, Terry Fox Medical Research Foundation, B.C., 1983-1991.

Appointed onto Faculty of the 'National Reference Service for Parasitology', May 1984-1999

Joined Faculty of Centre for Chinese Studies, Victoria, B.C., Oct 1984

Member of Board of Trustees and Executive Committee, The Biomedical Research Centre, UBC, Vancouver, B.C., 1986-1991.

Adjunct Professor, School of Public Health, University of North Carolina, Chapel Hill, N.C.

Panel Member – New Faculty and Sessional Lecturers Orientation, UVic, Sept. 1998.

Committee Member-UVic Alumni Teaching Awards, UVIC, Dec. 1999-Jan 2000

Instructor, UNESCO: Molecular Biology Course, International Cell Research Organization, Lagos, Nigeria, 2002

### **Most Significant Research Contributions**

Research in my laboratory has primarily been focused on cell surface molecules of pathogenic protozoan parasites (African trypanosomes and Leishmania), and on molecules of tsetse flies, the vector of African trypanosomes. In addition, our research is focused on derivation and characterization of monoclonal antibodies by novel methods and on protein microchemical approaches to protein discovery, including development of methods for analysis and quantitation of very small amounts of proteins, the latter being a limitation of current proteomics techniques.

- We showed that the variant surface glycoproteins (VSG) of bloodstream forms of trypanosomes have conserved framework regions, (contrary to prevailing models at the time), that surface-exposed epitopes are all topographically assembled (i.e. are not linear peptides) and that the variant-specific epitopes are all polypeptide, not CHO. Our work over a 10-year period thus contributed to the immunochemical structure of VSG's and the role of the structure in antigenic variation.

- We devised two diagnostic tests for human African sleeping sickness: one based on antibody detection, and the other on antigen detection which is more predictive of active infection. These can be used for a wider variety of species than was previously possible, in both East and West Africa.
- The major surface glycoprotein procyclin, found on insect stages of African trypanosomes, was discovered and characterized in my lab. This was followed by isolation and characterization of the analogs from several other species of trypanosomes. Structure-function studies continue in my lab and in many others in the U.S.A. and Europe.
- A novel form of programmed cell death (apoptosis) of African trypanosomes was characterized, in part, in my laboratory. This was the first description of apoptosis in single-celled eukaryotes, a process previously thought to occur only in metazoans. This work is now being pursued in my lab and in several others worldwide as a novel model system for studying apoptosis. Recently we published a paper showing that glycosylated forms of the parasite cell surface glycoprotein procyclin are receptors for ligands that cause the death of these parasites. This is relevant to the current proposal on trypanosome-tsetse interactions.
- A new method for derivation of monoclonal antibodies was invented and developed in my laboratory. The Clonacell-HY<sup>TM</sup> technology is a system for one-step, simultaneous selection and cloning of hybridomas. It is based on the use of a special semi-solid methylcellulose medium containing Bcell growth factors and selective chemicals. It is now marketed worldwide by StemCell Technologies Ltd. of Vancouver, B.C.
- Methods for identification and selection of high affinity anti-peptide monoclonal antibodies were developed and applied to large-scale reagent derivation for the analysis of the human proteome.

#### **Technology and product development**

- Developed a new method for derivation of monoclonal antibodies by a single-step selection and cloning procedure. This is marketed worldwide in 34 countries by StemCell Technologies Ltd, Vancouver, B.C.
- Developed a novel one-minute monoclonal antibody screening and isotyping system in collaboration with EY laboratories, San Mateo, CA. This 5<sup>th</sup>-generation colloidal gold-based system, Instant-Chek<sup>TM</sup>, will be sold internationally by StemCell Technologies, Vancouver, BC, starting in 2003.

#### **Patents**

- 1) Detection of gonococcal infections using monoclonal antibodies. Canadian Patent No. 1,220,147 (7 Apr.1987)\*
- 2) Detection of gonococcal infections using monoclonal antibodies. American Patent No. 4,755,459 (5 July 1988)\*

\*(co-inventor with Dr. Malcolm Perry, NRC, Ottawa)

### **Non-patented Inventions**

2. ClonaCell-HY™, a single-step selection and cloning system for hybridomas.  
(marketed by Stemcell Technologies, Inc., Vancouver, B.C.).
3. InstantChek™ isotyping kit for murine monoclonal antibodies (Marketed by EY Laboratories, San Mateo, CA and StemCell Technologies, Vancouver, B.C.).

### **RESEARCH GRANTS and FELLOWSHIPS**

#### **a. Research operating grants [currently held]**

<u>Agency</u>	<u>Title</u>	<u>Grant holders</u>	<u>Period (beg./end)</u>	<u>Amount awarded p/a to m</u>
<b><u>BCHRF</u></b>	Operating - Growth factors in protozoa	TWP	1992-94	\$25,000
<b><u>CIHR</u></b>	Operating - Peptide killing of trypanosomes	TWP	2004-07	\$99,180
	Operating - Mass spec diagnostics	TWP	2006-08	\$50,000
	International-Tsetse genomics and proteomics	TWP	2006-07	\$9,800
<b><u>DIFD</u></b>	Collaborative - Vaccine for Theileria	TWP, ILRI	2002-03	\$50,000
<b><u>IDRC</u></b>	Collaborative -Diagnosis of sleeping sickness.	TWP	1983-86	\$30,000
<b><u>MRC</u></b>	Operating (relinquished 2 yrs out of 3)	TWP	1987-88	\$40,000
<b><u>NATO</u></b>	Collaborative - Tsetse trypan interactions	TWP, IM	1990-92	\$6,300
	Collaborative - Tsetse trypan interactions	TWP, ML	1996-98	\$8,400
<b><u>NCI (US)</u></b>	RFA-Breast cancer diagnostics	MIT, TWP	2007-12	\$900,00
<b><u>NSERC</u></b>	Operating - Antigens of trypanosomes	TWP	1980-92	\$35,342
	Operating- Antigens of African trypanosomes	TWP	1992-00	\$75,000
	Research - Trypanosome-tsetse interactions	TWP	2000-06	\$75,000
	Discovery - Vector-trypan-host interactions	TWP	2007-12	\$73,500
	Collaborative - Interactions-parasites-vectors	TWP <i>et al.</i>	1996-99	\$78,000
	PRAI - Diagnosis of <i>Chlamydia/Neisseria</i>	TWP	1981-83	\$19,000
	Strategic - <i>Kudoa thyrsites</i> and soft-flesh	RWO, TWP	1999-01	\$163,63

<b>SPCA</b>	Hybridoma culture system	TWP	1995	\$1,736
<b>TIGR</b>	Mass spec analysis of gp96 peptides	TWP	2003-04	\$14,000
<b>UVIC</b>	Faculty Research Grants	TWP	1990-03	\$1,062
<b>WHO</b>	Operating - Diagnosis of sleeping sickness	TWP	1979-85	\$30,000

**b. Equipment grants**

<u>Agency</u>	<u>Title</u>	<u>Grant holders</u>	<u>Time period</u>	<u>Amount awarded</u>
<b>NSERC</b>	Dual CO <sub>2</sub> incubators	TWP	1988	\$15,000
<b>NSERC</b>	Bench top, continuous flow centrifuge	TWP	1991	\$17,360
<b>NSERC</b>	Orbital Environmental Shaker	TWP	1992	\$8,542
<b>NSERC</b>	Refrigerated microcentrifuge	TWP	1994	\$8,915
<b>NSERC</b>	Flow cytometer system (with BCFHRF)	TWP	1999	\$29,215
<b>NSERC</b>	2-D Zoom gel system	TWP	2003	\$18,000

**c. Honours, fellowships and scholarships**

1964-67	Government of British Columbia Scholarships
1965	Knights of Pythias Scholarship
1966	IBM Scholarship
1966	University of British Columbia Board of Governors Special Award
1967	Canadian Medical Research Council Scholarship
1970-73	Canadian Medical Research Council Trainee
1995	Inaugural Faculty of Science Excellence in Teaching Award (UVic)
2004	Wardle Award for contributions to Canadian Parasitology (Can Soc for Zoology)

**Contributions to the Training of Highly Qualified Personnel**

(I am or was the sole supervisor of the people listed below)

**a) Undergraduate Students**

(All students worked on immunochemistry or protein chemical aspects of African trypanosomes and tsetse flies)

Saini, Manik	2001 - 2002	currently MSc student Lon. S. Trop Med
Hunter, Michael	2000 - 2001	currently fourth year Biochemistry, New York
Hartwig, Rhannon	1999, Sept-Dec.	currently fourth year undergrad-Biochem
Hache, Nancy	1999, May-Sept	currently MSc student, Univ. of New Brunswick
Serink, Jennifer	1999, Jan-Apr.	currently at dental school - UBC, Vancouver



Eaves, Sarah	1998, May-Aug	currently MSc student -University of Alberta
Laszczak, Mario	1998, Jan-Aug	currently MSc Bioinformatics – UVic
Wagner, Mary	1998-1999	currently PhD student, Bioc. Univ of Victoria
Wilfred Jefferies	1979-1981	currently Professor, UBC Biomedical Research
Chrystal McNabb	1982-1985	currently admin officer, Genome BC, Vancouver

#### b) Graduate Students & Technicians

Angela Jackson	2002-present	MSc ( <i>Theileria parva</i> vaccine)
Emily Jansen	2002-present	MSc (trypanosome surface)
Jody D. Haddow	1999-present	PhD (tsetse-tryp interactions)
Lee R. Haines	1999-present	MSc (tsetse-tryp interactions)
Neeloffer Mookherjee	1995-2001	PhD (trypanosome surface molecules)
Jennifer C. Chase	1999-2001	Research Technicia
Morag H. Stewart	2000-2001	Research technician
Michael A. Bridge	1996-1999	MSc (trypanosome surface molecules) Currently medical resident-Halifax
Robert P. Beecroft	1983-1997	Research Technician now President of ImmunoPrecise Antibodies, Ltd., Victoria, B.C., Canada
Caroline E. Cameron	1990-1996	PhD (trypanosome surface molecules)
Cory Tuckey	1994-1996	MSc, Now Scientist-NEB, Beverly MA
Doug Tolson	1987-1991	PhD. Now IDC Officer, UVIC
Margaret Liu	1986-1980	PhD Now Faculty, Ann Arbor MI
Jennifer Richardson	1979-1983	PhD Now Faculty, Paris, France

Leslie Mitchel

1980-84

PhD Now Faculty, Jerusalem, Israel

**Seminars and Presented Papers**

- Sep. 1974      The immune response to ferredoxin peptides.  
Department of Pathology, Cambridge, England
- Oct. 1975      Cell-mediated immunity to methylcholanthrene-induced murine sarcomas. MRC Laboratory of Molecular Biology, Cambridge, England
- Sep. 1976      What is the genetic origin of the antigenic diversity of methylcholanthrene-induced murine sarcomas?  
Middlesex Hospital Medical School, Mill Hill, London, England
- June 1977      Cytotoxic T lymphocyte killing of influenza virus-infected lymphoma cells. National Institute for Medical Research, Mill Hill, London, England.
- June 1977      A myeloma-hybrid producing antibody specific for an allotype on "IgD-like" molecules of the mouse. Department of Genetics, University of Cologne, Germany
- June 1977      A myeloma-hybrid producing antibody specific for an allotype on "IgD-like" molecules of the mouse. German Cancer Research Centre, Heidelberg, Germany
- July 1977      A myeloma-hybrid producing antibody specific for an allotype on "IgD-like" molecules of the mouse. Basel Institute for Immunology, Basel, Switzerland
- Sept. 1977      Production of monoclonal antibodies by cell fusion. Biochemical Discussion Group, University of British Columbia, Vancouver
- Dec. 1977      Production by cell fusion of monoclonal antibodies of predefined specificity. University of Nairobi, Nairobi, Kenya
- June 1978      Immune suppression in experimental African trypanosomiasis. MRC Laboratory of Molecular Biology, Cambridge, England
- June 1978      Suppressor cells in *Trypanosoma congolense*-infected mice. 6th International Congress on Lymphatic Tissues and Germinal Centres in Immune Reactions. Kiel, West Germany

- July 1978 Immune depression and suppression in African trypanosomiasis. Department of Pathology, Justus Leibzig University, Giessen, Germany
- Aug. 1978 Suppression of immune responses in trypanosome-infected mice. Department of Immunology, Aba Khoushy School of Medicine, Technion, Haifa, Israel
- Feb. 1980 Monoclonal antibodies and their application to the study of parasitology. International Laboratory for Research on Animal Diseases, Nairobi, Kenya
- Mar. 1980 The immunology of African trypanosomiasis. Dept. of Immunology, University of Toronto, Ontario, Canada
- Mar. 1980 Antigenic variation in African trypanosomes. Ontario Cancer Institute, Toronto, Ontario, Canada
- Apr. 1980 Immunology and Nutrition. University Extension, University of Victoria (evening classes): Recent Advances in Medicine. Victoria, B.C., Canada
- July 1980 Biotechnology and its application: the significance to B.C. Discovery Parks. University of Victoria, meeting with the Capital Regional Economic Development Council. Victoria, B.C., Canada
- Sept. 1980 Biotechnology and its application: the significance to B.C. Discovery Parks. University of Victoria, meeting with the Capital Regional Economic Development Council. Victoria, B.C., Canada
- Sept. 1980 Changes in the immune system associated with experimental African trypanosomiasis. University of Victoria, Department of Biochemistry and Microbiology, Seminar Program, Victoria, B.C., Canada
- Feb. 1981 Parasites and Health in the tropical third world. Lester B. Pearson College of the Pacific, Victoria, B.C., Canada
- Apr. 1981 Changes in the immune system associated with experimental African trypanosomiasis..Amer.Soc Trop Med Hygiene. Atlanta, Georgia, U.S.A.
- Apr. 1981 Detection of common antigenic components in trypanosome populations. Prince Leopold Institute, Antwerp, Belgium

- Oct. 1981            Monoclonal antibodies, their properties and use in studying disease. WHO meeting on tropical diseases. University of Singapore, Singapore
- Oct. 1981            Techniques for isolation and characterization of antigens from complex mixtures. University of Singapore, Singapore
- Oct. 1981            Monoclonal antibodies and disease: Future outlook and novel approaches. University of Singapore, Singapore
- Dec. 1981            The use of monoclonal antibodies in immunoparasitology. Ortho Symposium on Hybridomas. Toronto, Ontario, Canada
- Feb. 1982            The immunobiology of African sleeping sickness. University of Victoria, Department of Biology, Victoria, B.C., Canada
- Mar. 1982            Cell fusion and the production of tailor-made antibodies. Lester B. Pearson College of the Pacific, Victoria, B.C., Canada
- Mar. 1982            Monoclonal antibodies and associated technologies: Applications to biological and biochemical research. National Research Council, Ottawa, Ontario, Canada
- Apr. 1982            Modern immunochemical methods in the study of parasite antigens. Five lectures at the University of North Carolina, Chapel Hill, N.C., U.S.A.
- Aug. 1982            Immunity to African trypanosomes. Armaur Hansen Institute, Addis Ababa, Ethiopia
- Oct. 1982            African trypanosomes: Studies on the major surface glycoprotein antigen. University of British Columbia, Department of Physiology, Vancouver, B.C., Canada
- Nov. 1982            Analysis of complex biochemical mixtures using new advances in biotechnology. Universities Council of B.C., Outlook Conference V. Laurel Point Inn, Victoria, Canada
- June 1983            The evolution of a scientist in a non-scientific society. Trinity Western College, Surrey, B.C., Canada
- Oct. 1983            Immunochemistry of trypanosome antigens. Biomembranes Group Annual Meeting. Banff, Alberta, Canada
- Nov. 1983            Monoclonal antibodies. British Columbia Society of Medical Technicians. Nanaimo, B.C., Canada

- Nov. 1983                   Antigens on various life-cycle stages of African trypanosomes.  
Yale University, New Haven, Connecticut, U.S.A.
- May 1984                   Immunobiology of African trypanosomes.  
Department of Pathology, Shaughnessy Hospital, Vancouver, B.C.,  
Canada
- June 1984                   Monoclonal antibodies and recombinant DNA.  
Canadian Society for Medical Laboratory Technicians Annual  
Meeting. Vancouver, B.C., Canada
- Dec. 1984                   Immunology and ethics. Canadian College for Chinese Studies,  
Victoria, B.C., Canada
- Feb. 1985                   Monoclonal antibodies and disease diagnosis. Helmcken Hospital,  
Victoria, B.C., Canada
- Aug. 1985                   The structure and immunochemistry of variant surface antigens of  
African trypanosomes. ILRAD, Nairobi, Kenya
- Jun. 1986                   Genetic recombination and species discrimination in African  
trypanosomes: 2-D gel analysis. ILRAD, Nairobi, Kenya
- Oct. 1986                   African trypanosomes: adaptive strategies for survival in hostile  
environments of different hosts. Symposium of Biological  
Implications of Pathogenicity. Lake Louise, Alberta, Canada
- Nov. 1986                   How to care for your new monoclonal antibodies.  
BIOFOR Second Annual Meeting. Pacific Forestry Centre,  
Victoria, B.C., Canada
- Nov. 1986                   Biotechnology. Cedar Hill Junior Secondary School, Victoria, B.C.,  
Canada
- Feb. 1987                   The Tao of immunology. Canadian College for Chinese Studies,  
Victoria, B.C., Canada
- Jun. 1987                   Procyclin. Canadian Society for Immunology, Winnipeg, Manitoba,  
Canada
- Oct. 1987                   Procyclin: a trypanosome antigen important for diagnosis and  
vaccination. Canadian Society of Microbiologists Annual Meeting,  
Western Branch, Empress Hotel, Victoria, B.C., Canada
- Mar. 1988                   Third World Diseases. Gulf Island Secondary School, Ganges,  
Saltspring Island, B.C., Canada

- Apr. 1988 New strategies for development of vaccines. Medicine Rounds. Helmcken Hospital, Victoria, B.C., Canada
- Apr. 1988 The immunochemistry of trypanosome VSG's of the WaTat serodeme. West Coast Trypanosome Meeting. Asilomar, California, U.S.A.
- Jan. 1989 Surface antigens of trypanosomes during differentiation. Department of Microbiology, University of British Columbia, Vancouver, B.C., Canada
- Feb. 1989 Procyclin and transmission blocking. CSI Annual Meeting. Lake Louise, Alberta, Canada
- Feb. 1989 Procyclin and VSG changes during trypanosome differentiation. Department of Biochemistry, University of Kentucky, Lexington, Kentucky, U.S.A.
- June 1989 Research priorities. Ministry of Health Cabinet Rooms, Vancouver, B.C., Canada
- Sep. 1989 Immunochemistry of trypanosome surface antigens. West Coast Molecular Parasitology Meeting. Point-no-point, Sooke, B.C.
- Oct. 1989 The great neglected diseases of mankind: Vaccination and immunodiagnosis. B.C. Society of Medical Technologists evening lecture series. Victoria, B.C., Canada
- Mar. 1990 The immunobiology of African trypanosomes. Fourth Annual Spring Meeting of the Canadian Society for Immunology. Mt. St. Gabriel, Québec, Canada
- Apr. 1990 Why it is important to study parasitic diseases. University of Victoria Medical Biosafety Meeting. Dunsmuir Lodge, Victoria, B.C., Canada.
- Sep. 1990 The Lipophosphoglycan of *Leishmania donovani*. ILRAD, Nairobi, Kenya
- Dec. 1990 Growth factors in African trypanosomes. ILRAD, Nairobi, Kenya
- Jan. 1991 Immunity to *Leishmania donovani* - the role of Lipophosphoglycan. Swiss annual trypanosomiasis meeting, Les Diablerets, Switzerland.
- Sept. 1991 Studies on a *Leishmania donovani* protective antigen. ILRAD, Nairobi, Kenya

- Sept. 1991 Trypanosome procyclins. ILRAD, Nairobi, Kenya
- Mar. 1992 A procyclin analog in *Trypanosoma congolense*. Canadian Society for Immunology, Mt. St. Gabriel, Quebec
- Sept. 1992 A procyclin analog in *Trypanosoma congolense*. Molecular Parasitology Meeting, Woods Hole, Massachusetts
- Oct. 1992 Vaccines for tropical diseases: recent advances. B.C. Society for Med Lab Techs., Helmcken Hospital, Victoria, B.C. Canada
- Dec. 1992 Membrane molecules of African trypanosomes. New York University Medical Center, New York, New York
- Oct. 1993 Surface molecules of African Trypanosomes and *Leishmania*, Institute for Parasitology, University of Bern, Bern, Switzerland.
- Nov. 1993 Major Surface Glycoproteins on procyclic stage African trypanosomes, MacArthur Foundation. Hamilton Park, Newark, N.J. USA
- Mar. 1994 Trypanosome surface glycoproteins - tsetse fly interactions. Canadian Society for Immunology, 7th Annual Spring Meeting, Mt. St. Gabriel, Quebec.
- Mar. 1994 The procyclin coat of African trypanosomes. Dept. of Immunology, University of Toronto, Toronto, Ontario.
- June 1994 Major Surface Glycoproteins of African trypanosomes. Dept. of Pathology and Lab Medicine, Medical Microbiology, UBC, Vancouver, B.C., Canada
- June 1995 The major surface glycoproteins of insect stage African trypanosomes: the parasite-tsetse fly interface. Dept. of Biological Sciences, University of Alberta, Edmonton, AB, Canada.
- Feb. 1996 Games parasites play. Biotechnology Laboratory, UVC, Vancouver, B.C. Canada
- Mar. 1996 The major surface glycoproteins of African trypanosomes: the parasite-tsetse fly interface. Dept. of Biochemistry and Microbiology, University of Victoria, Victoria, B.C., Canada.

- Nov. 1996 One hundred years of African sleeping sickness: towards molecular strategies for disease intervention. University of Namibia, Windhoek, Namibia.
- Feb. 1997 The major surface glycoproteins of insect forms of African trypanosomes. ILRI, Nairobi, Kenya.
- Mar. 1997 Molecular interactions between procyclic trypanosomes and the tsetse fly: new strategies for parasite control. Kenya Trypanosomiasis Research Institute, Muguga, Kenya.
- Jul. 1997 Programmed cell death in procyclic African trypanosomes. ILRI, Nairobi, Kenya.
- Dec. 1998 Procyclin Isoforms and tsetse interactions. Univ. of Glasgow. Glasgow, Scotland
- Oct. 1998 Procyclins and the trypanosome – tsetse interface. Univ. of Saskatchewan. Saskatoon, Saskatchewan, Canada
- Oct. 1998 The importance of basic research for long-term development. Canadian College for Chinese Medicine, Victoria, B.C., Canada
- Apr. 1999 Basic Research: A necessary prelude to application and development in science. Canadian College for Chinese Medicine, Victoria, B.C., Canada
- Sep. 1999 Teaching tips from a dubious award winner. Learning and Teaching Centre Orientation for New Instructors. UVIC, Victoria, B.C., Canada.
- Nov. 1999 The Sleeping Dragon: One Hundred Years of African Sleeping Sickness. Dean's Lecture Series. UVIC Continuing Studies. Victoria.
- Feb. 1999 What use is a newborn baby? Why basic research in immunology is important. Biochemistry and Microbiology Department–Luncheon Seminar. UVIC.
- May 1999 Procyclins of African trypanosomes-ligands for induction of cell death. Johns Hopkins School of Medicine. Baltimore, MD.
- May 1999 A unique form of cell death in African trypanosomes. Yale University School of Medicine, New Haven, Connecticut.
- Dec. 2000 If you can't get rid of the parasites, get rid of the parasitologists: 100 years of African sleeping sickness. Symposium in honour of Dr. Doug Kilburn, my PhD supervisor. UBC, Vancouver.
- May 2001 African Sleeping Sickness. Mount Douglas High School, Victoria
- Nov. 2001 Twenty-five years of monoclonal antibodies. CCAC, Vancouver
- Dec. 2001 The proteome of *Theileria parva*. ILRI, Nairobi, Kenya.



- Aug 2002 *Theileria parva* -a model for cell transformation. UVIC Dept of Biochemistry, Victoria, BC
- Sept 2002 Tsetse - trypanosome interactions Biomedical Research Center, UBC Vancouver.
- Sept 2002 *Theileria parva*, a parasite that causes irreversible transformation of T lymphocytes. Department of Microbiology, UBC, Vancouver.
- Oct 2002 Identification of midgut molecules of tsetse flies using protein microchemistry and mass spectrometry. American Society for Tropical Medicine and Hygiene Annual Meeting. Denver, Colorado.
- Oct 2003 Molecular interactions between African trypanosomes and their tsetse fly vectors: strategies for interference with parasite transmission. McGill Institute of Parasitology, Montreal, Quebec.
- Mar 2004 Tropical Diseases and the shaping of the African continent. WUSC, Victoria, B. C.
- May 2004 One hundred years of African sleeping sickness: the long journey of the African trypanosome; Wardle Award Lecture Acadian University, Wolfville, Nova Scotia
- Aug, 2004 Midgut and salivary gland molecules of the tsetse fly *Glossina morsitans morsitans* Obihiro University, Hokkaido, Japan.
- Nov 2004 Genetic modification of tsetse flies for control of African sleeping sickness. Centre for Biomedical Research, UBC, Vancouver, B.C.
- Nov 2004 Genetic modification of tsetse for control of African sleeping sickness. Department of Biological Sciences, University of Alberta, Edmonton, Alberta.
- Mar 2005 Genetic modification of tsetse flies for control of African sleeping sickness. Department of Biology, Simon Fraser University, Vancouver, B.C.
- Mar 2005 Immunochemical and protein chemical approaches to identification of proteins involved in host-parasite and vector-parasite interactions. Africa Genome Plenary lecture, Nairobi, Kenya.
- May 2006 Measuring biomarker candidates by antibody enrichment and targeted mass spectrometry. Canary Foundation Symposium-Early Detection of Cancer, San Jose California.
- Dec, 2006 Molecular Interactions involved in transmission of African trypanosomes by their tsetse vectors. UBC Immunology, Vancouver. B.C.

- May, 2007 Antibody enrichment of Peptides for SISCAPA. Canary Foundation Early Detection of Cancer. Stanford University, Palo Alto, California.
- Sept 2007 Progress in antibody development for peptide diagnostic assays. Korean Biotechnology Institute SISCAPA Symposium, Daejeon, Korea.
- Sept 2007 Antibodies for peptide enrichment in biomarker qualification assays. HUPO Annual Meeting, Seoul, Korea
- Oct 2007 Antibodies for peptide enrichment in biomarker qualification assays. National Cancer Institute CPTAC meeting on cancer diagnosis- Ann. Meeting Rockville, MD
- Oct 2007 Development of new medical diagnostics using antibodies and Mass spectrometry. UBC Microbiology Dept. Immunology lecture series.
- Nov 2007 Selection of surrogate peptides and production of rabbit anti-peptide monoclonal antibodies (RabMabs) of high affinity for peptide enrichment. NCI-CPTAC retreat for project assessment-annual meeting. Tucson, Arizona
- Jan 2008 Antibodies for peptide enrichment in biomarker qualification. Proteomics Workshop: Partnership for Personalized Medicine Phoenix, Arizona
- Jan 2008 Antibodies for peptide enrichment in biomarker qualification. UVic- Genome BC Proteomics Centre Symposium on Immunoproteomics. UVic, Victoria, BC
- Feb 2008 New Diagnostic Methods using Antibodies and mass spectrometry. Life Sciences Institute In-house seminars, UBC Vancouver, BC
- Jun 2008 Antibodies for peptide enrichment in biomarker qualification assays Bioforum Shanghai, Shanghai, China
- Jun 2008 Antibodies for peptide enrichment in biomarker qualification assays.Epitomics Inc., Hangzhou, China
- Jul 2008 Analysis of host-trypanosome-vector interactions using proteomics technologies. Proteomics of Infectious diseases meeting. Population Health Metrics Research Consortium Univ of Washington, Seattle, WA, USA
- Aug 2008 Development of new medical diagnostics using antibodies and mass spectrometry. Rising Stars Program for new Graduate students. UVic University Club, Victoria

Oct 2008	Peptides associated with affinity purified antibodies-a problem affecting antibody capture efficiency. CPTAC biannual review meeting, Broad Institute MIT/Harvard, Cambridge, MA, USA
April 2009	Anti-peptide antibodies for proteomics. European Science Foundation Meeting on Affinity Proteomics. Alpbach, Austria.
May 2009	A human proteome detection and quantitation project (hPDQ) for blood cells. Canadian Proteomics Initiative Workshop, Vancouver BC
June 2009	Differential protein expression throughout the life cycle of <i>Trypanosoma congolense</i> . Symposium of Zoonotic Diseases, Obihiro University, Obihiro Japan,

### **Articles Published in Refereed Journals**

1. Pearson, T., J. Levy and D. Kilburn, 1975. The effect of specific cell inactivation on the cellular immune response to ferredoxin peptides. *Eur. J. Immunol.* 5, 65-69.
2. Pearson, T., J. Levy, D. Kilburn, and M. Fairhurst, 1976. The effect of antigen suicide on numbers of cells binding defined antigenic determinants. *J. Immunol. Methods* 10, 99-104.
3. Kohler, G., T. Pearson, and C. Milstein, 1977. Fusion of T and B cells. *Somatic Cell Genetics* 3, 303-312.
4. Pearson, T., G. Galfre, A. Ziegler, and C. Milstein, 1977. A myeloma hybrid-producing antibody specific for an allotypic determinant of "IgD-like" molecules of the mouse. *Eur. J. Immunol.* 7, 684-690.
5. Pearson, T., K. Sikora, and E. Lennox, 1978. Measurement of H-2 antigen and immunogenicity of methylcholanthrene-induced murine sarcomas. *Br. J. Cancer* 37, 530-535.
6. Pearson, T., G. Roelants, L. Lundin, and K. Mayor-Withey, 1978. Immune depression in trypanosome-infected mice. I. Depressed responses. *Eur. J. Immunol.* 8, 723-727.
7. Pearson, T., G. Roelants, L. Lundin, and K. Mayor-Withey, 1979. The bovine lymphoid system: binding and stimulation of peripheral blood lymphocytes by lectins. *J. Immunol. Methods* 26, 271-282.
8. Roelants, G., T. Pearson, H. Tyrer, L. Lundin, and K. Mayor-Withey, 1979. Immune depression in trypanosome-infected mice. II. Characterization of the cell types involved. *Eur. J. Immunol.* 9, 195-199.
9. Pearson, T., G. Roelants, L. Lundin, and K. Mayor-Withey, 1989. Immune depression in trypanosome-infected mice. III. Suppressor cells. *Eur. J. Immunol.* 9, 200-204.

10. Roelants, G.E., T.W. Pearson, W.I. Morrison, L.B. Lundin and K.S. Mayor-Withey, 1979. Immune depression in trypanosome-infected mice. IV. Kinetics of suppression and its reversal by the trypanocidal drug Berenil. *Clin. Exp. Immunol.* 37, 457-469.
11. Pearson, T.W. and N.L. Anderson, 1980. Dissection of complex antigen mixtures using monoclonal antibodies and two-dimensional gel electrophoresis. *Anal. Biochem.* 101, 377-383.
12. Pearson, T.W., T.T. Dolan, D.A. Stagg and L.B. Lundin, 1979. Cell mediated immunity to *Theileria*-transformed cell lines. *Nature* 281, 678-680.
13. Pearson, T.W., M. Pinder, G.E. Roelants, S.K. Kar, L.B. Lundin, K.S. Mayor-Withey and R.S. Hewett, 1980. Methods for derivation and detection of anti-parasite monoclonal antibodies. *J. Immunol. Methods* 34, 141-154.
14. Pinder, M., T.W. Pearson, G. Roelants and K.S. Mayor-Withey, 1980. The bovine lymphoid system. II. Derivation and partial characterization of monoclonal antibodies against bovine peripheral blood lymphocytes. *Vet. Immunol. Immunopath.* 1, 303-316.
15. Pearson, T.W., S. Kar, T. McGuire and L. Lundin, 1981. Trypanosome surface antigens: studies using monoclonal antibodies and two-dimensional gel electrophoresis. *J. Immunol.* 126, 823-828.
16. Kar, S., G. Roelants, K. Mayor-Withey and T.W. Pearson, 1981. Immunodepression in trypanosome-infected mice. V. Comparison of immune responses of different lymphoid organs, *Eur. J. Immunol.* 11, 100-105.
17. Masake, R.A., T.W. Pearson, P. Wells and G. Roelants, 1981. The *in vitro* response to mitogens of leukocytes from cattle infected with *Trypanosoma congolense*. *Clin. Exp. Immunol.* 43, 583-589.
18. Anderson, N.L., S. Nance, T.W. Pearson and N.G. Anderson, 1982. Analytical techniques for cell fractions. XXIX. Specific antiserum staining of two-dimensional electrophoretic patterns of human plasma proteins immobilized on nitrocellulose. *Electrophoresis* 3, 135-142.
19. Pearson, T.W., L. Saya, P. Howard and T. Buckley, 1982. The use of aerolysin toxin as an aid for visualization of low numbers of African trypanosomes in whole blood. *Acta Tropica* 39, 73-77.
20. Pearson, T.W., R. Hewett, G.E. Roelants, D.A. Stagg and T.T. Dolan, 1982. Studies on the induction and specificity of cytotoxicity to *Theileria*-transformed cell lines. *J. Immunol.* 128, 2509-2513.

21. Mitchell, L.A. and T.W. Pearson, 1983. Antibody responses induced by immunization of inbred mice susceptible and resistant to African trypanosomes. *Infect. and Immun.* 40, 894-902.
22. Nagai, T., T.W. Pearson, F. Peng, E.G. McGeer and P.L. McGeer, 1983. Immunohistochemical staining of the human forebrain with monoclonal antibody to human choline acetyltransferase. *Brain Research* 265, 300-306.
23. Chart, H., T.W. Pearson and T.J. Trust, 1984. Detection of specific fish antibody using an inhibition enzyme-linked immunosorbent assay (inhibition-ELISA). *J. Immunol. Methods* 68, 19-24.
24. Olafson, R.W., M.W. Clarke, T.W. Pearson, A.F. Barbet and T.C. McGuire, 1984. Amino-terminal sequence homology among variant surface glycoproteins of African trypanosomes. *Mol. Biochem. Parasitol.*, 12, 287-298.
25. Clarke, M.W., R.W. Olafson and T.W. Pearson, 1984. Purification of major variable surface glycoproteins from African trypanosomes by reverse-phase high performance liquid chromatography. *Anal. Biochem.* 142, 360-368.
26. Parish, N.M., W.I. Morrison and T.W. Pearson, 1985. Identification of an antigen specific to *Trypanosoma congolense* using monoclonal antibodies. *J. Immunol.* 134, 593-597.
27. Anderson, N.L., N.M. Parish, J.P. Richardson and T.W. Pearson, 1985. Comparison of African trypanosomes of different antigenic phenotypes, subspecies and life cycle stages by two-dimensional gel electrophoresis. *Mol. Biochem. Parasitol.* 16, 299-314.
28. Clarke, M.W., R.W. Olafson and T.W. Pearson, 1985. Rapid preparative scale purification of myristylated variant surface glycoproteins from African trypanosomes. *Mol. Biochem. Parasitol.* 17, 19-34.
29. Richardson, J.P., L. Jenni, R.P. Beecroft and T.W. Pearson, 1986. Procyclic tsetse fly midgut forms and culture forms of African trypanosomes share stage- and species-specific surface antigens identified by monoclonal antibodies. *J. Immunol.* 136, 2259-2264.
30. Mitchell, L.A., T.W. Pearson and J. Gauldie, 1986. Interleukin-1 and Interleukin-2 production in resistant and susceptible mice infected with *Trypanosoma congolense*. *Immunology* 57, 291-296.
31. Mitchell, L.A. and T.W. Pearson, 1986. Antibody responses in resistant and susceptible inbred mice infected with *Trypanosoma congolense*. *Immunology* 57, 297-303.
32. Pearson, T.W., M. Liu, I.C. Gardiner, D. Longridge, P.D. Sayer, S.S. Gould, J.N. Waitumbi and A.R. Njogu, 1986. Use of procyclic trypanosomes for detection of

- antibodies in sera from vervet monkeys infected with *Trypanosoma rhodesiense*: an immunodiagnostic test for African sleeping sickness. *Acta Tropica* 43, 391-399.
33. Gardiner, P.R., T.W. Pearson, M.W. Clarke, and L.M. Mutharia, 1987. Identification and isolation of a variant surface glycoprotein from *Trypanosoma vivax*. *Science* 235, 774-777.
  34. Mutharia, L.M., and T.W. Pearson, 1987. Surface carbohydrates of procyclic culture forms of African trypanosomes studied using FACS analysis and agglutination with lectins. *Mol. Biochem. Parasitol.* 23, 165-172.
  35. Clarke, R.W., A.F. Barbet, and T.W. Pearson, 1987. Structural features of antigenic sites on variant surface glycoproteins from *Trypanosoma brucei*. *Mol. Immunol.* 24, 707-713.
  36. Liu, M.K. and Pearson, T.W., 1987. Detection of circulating trypanosomal antigens by double antibody ELISA using antibodies to procyclic trypanosomes. *Parasitology* 95, 77-290.
  37. Pearson, T.W., S.K. Moloo, and L. Jenni, 1987. Culture form and tsetse fly midgut form procyclic *Trypanosoma brucei* express common proteins. *Mol. Biochem. Parasitol.* 25, 273-278.
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  39. Liu, M.K., T.W. Pearson, P.D. Sayer, S.S. Gould, J.N. Waitumbi and A.R. Njogu, 1988. Detection of circulating antigens in vervet monkeys infected with *Trypanosoma brucei rhodesiense*: a sensitive antigen trapping ELISA for immunodiagnosis of African sleeping sickness. *Acta Tropica* 45, 321-330.
  40. Richardson, J.P., R.P. Beecroft, D.L. Tolson and T.W. Pearson, 1988. Procyclin: an unusual immunodominant glycoprotein surface antigen from the procyclic stage of African trypanosomes. *Mol. Bioc. Parasitol.* 31, 203-216.
  41. Liu, M.K., P. Cattand, I.C. Gardiner and T.W. Pearson, 1989. Immunodiagnosis of sleeping sickness due to *Trypanosoma brucei gambiense* by detection of anti-procyclic antibodies and trypanosome antigens in patients' sera. *Acta Tropica* 46, 257-266.
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47. Sugimoto, C., L.M. Mutharia, P.A. Conrad, T.T. Dolan, W.C. Brown and T.W. Pearson, 1989. Protein changes in bovine lymphoblastoid cells induced by infection with the intracellular parasite *Theileria parva*. *Mol. Biochem. Parasitol.* 37, 159-170.
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(plus 9 abstracts published in 2008-09)

“END”

## Appendix B

1-43. (Canceled)

44. (Previously Presented) A method of quantifying an amount of at least a first monitor peptide and a second monitor peptide in a biological sample, comprising:  
contacting the sample with

- (i) a first anti-peptide antibody specific for said first peptide and;
  - (ii) a known quantity of a labeled version of said first peptide;
- contacting the sample with
- (i) a second antipeptide antibody specific for said second peptide, wherein said second antibody is different from said first antibody and;
  - (ii) a known quantity of a labeled version of said second peptide, separating peptides bound by said first and said second antibodies from unbound peptides;
- eluting said peptides bound by said first and said second antibodies from said antibodies;
- measuring the amount of said first peptide eluted from said first antibody using a mass spectrometer;
- measuring the amount of said labeled version of said first peptide eluted from said first antibody using a mass spectrometer;
- calculating the amount of the first peptide in the biological sample;
- measuring the amount of said second peptide eluted from said second antibody using a mass spectrometer;
- measuring the amount of the labeled version of the second peptide eluted from said second antibody using a mass spectrometer; and
- calculating the amount of the second peptide in the biological sample, wherein said biological sample is a proteolytic digest of a bodily fluid sample.

45-47. (Canceled)

48. (Previously Presented) The method of claim 44, wherein at least one of said first and said second antibodies is a monoclonal antibody.

49. (Previously Presented) The method of claim 44, wherein at least one of said first and said second antibodies is a polyclonal antibody.

50. (Previously Presented) The method of claim 44, wherein said first and said second antibodies are both polyclonal antibodies.

51. (Previously Presented) The method of claim 44, wherein said first and said second antibodies are both monoclonal antibodies.

52-53. (Canceled)

54. (Previously Presented) The method of claim 44, wherein the labeled version of the first peptide includes at least one site at which a stable isotope is substituted for the corresponding predominant natural isotope in more than 98% of peptide molecules.

55. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to a support.

56. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to a packed column.

57. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to a monolithic porous support.

58. (The method of claim 44, further comprising: attaching the first antibody to a mesh.

59. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to magnetic beads.

60. (Previously Presented) The method of claim 44, wherein the first peptide and the second peptide are selected from among the set of peptides produced by digestion of the target protein to provide high signal to noise in the mass spectrometer.

61. (Previously Presented) A method for quantifying the amount of a peptide, comprising: contacting the sample with

- (i) an anti-peptide antibody specific for said peptide;
- (ii) a known quantity of a labeled version of the peptide, separating peptides bound by said antibody from unbound peptides eluting said peptide bound by said antibody from said antibody; measuring the amount of the peptide eluted from said antibody using a mass spectrometer; and calculating the amount of the peptide in the biological sample; wherein said biological sample is a proteolytic digest of a bodily fluid.

62-63. (Canceled)

64. (Previously Presented) The method of claim 61, further comprising: preparing the labeled version of the peptide.

65. (Previously Presented) The method of claim 61, wherein the labeled version of the peptide includes at least one site at which a stable isotope is substituted for the predominant natural isotope in more than 98% of peptide molecules.

66-70. (Canceled)

71. (Currently Amended) The method of claim 44, further comprising: preparing the labeled version of the monitor peptide.

72. (Currently Amended) The method of claim 71, wherein the labeled version of the monitor peptide includes a stable isotope.

73. (Canceled).

74. (Previously Presented) method of claim 44, wherein said first anti-peptide antibody is created using said first peptide or a nonmaterially modified version of the first monitor peptide.

75. (Previously Presented)) The method of claim 44, further comprising: creating the first antibody using the first peptide or a non-materially modified version of the first peptide.

76. (Canceled).

77. (Previously Presented) The method of claim 61, further comprising: creating the anti-peptide antibody using the peptide or a non-materially modified version of the peptide.

78. (Currently Amended) The method of claim 44, wherein the said bound peptides are subjected to a chromatography step after elution from said antibodies and before introduction into said mass spectrometer.

79-80. (Canceled)

81. (Currently Amended) The method of claim 61, wherein said bound peptides are subjected to a chromatography step after elution from said antibody and before introduction into said mass spectrometer.

82. (Previously Presented) The method of claim 61, wherein the anti-peptide antibody is a polyclonal antibody.

83. (Previously Presented) The method of claim 61, wherein the anti-peptide antibody is a monoclonal antibody.

84. (Previously Presented) The method of claim 44 wherein said first and second peptides are proteolytically cleaved from first and second sample proteins, respectively, and wherein the amounts of said first and second proteins in said body fluid sample are calculated from the amounts of said first and said second peptides in the sample.

85. (Previously Presented) The method of claim 61 wherein said first and second peptides are proteolytically cleaved from first and second sample proteins, respectively, and wherein the amounts of said first and second proteins in said body fluid sample are calculated from the amounts of said first and said second peptides in the sample.

86. (Previously Presented) The method of claim 61, wherein the polyclonal antibody is created using the monitor peptide or a non-materially modified version of the monitor peptide.